

09/979518

WHAT IS CLAIMED IS:

1. A purified thermostable template-dependent DNA polymerase from the species *Bacillus stearothermophilus* comprising reverse transcriptase activity in the presence of magnesium ions at a concentration of at least 1 mM and in the substantial absence of manganese ions.
2. The polymerase of claim 1, wherein said polymerase is type strain 5 ATCC 12980.
3. The polymerase of claim 1 or 2, further comprising 3'-5' exonuclease activity and 5'-3' exonuclease activity.
4. The polymerase of claim 3, wherein said polymerase is modified or mutated to reduce or eliminate 3'-5' exonuclease activity.
5. The polymerase of claim 3, wherein said polymerase is modified or mutated to reduce or eliminate 5'-3' exonuclease activity.
6. The polymerase of claim 5, wherein said polymerase has a molecular weight between about 55 to about 65 kilodaltons as determined by SDS polyacrylamide electrophoresis.

7. The polymerase of claim 1 or 2, wherein said magnesium ion concentration is about 1 mM to about 10 mM.

8. The polymerase of claim 1 or 2, wherein the source of said magnesium ions is a magnesium-containing buffer.

9. The polymerase of claim 1 or 2, wherein the source of said magnesium ions is a magnesium-containing salt.

10. The polymerase of claim 9, wherein said magnesium-containing salt is selected from the group consisting of magnesium chloride, magnesium sulfate and magnesium acetate.

11. A method of preparing one or more cDNA molecules from one or more RNA templates, comprising

- (a) mixing one or more RNA templates with one or more of the polymerases of claim 1 or claim 2 to form a mixture; and
- (b) incubating said mixture under conditions sufficient to synthesize one or more cDNA molecules complementary to all or a portion of said one or more templates.

12. The method of claim 11, wherein said incubating step (b) comprises incubating said mixture at a temperature and for a time sufficient to make a DNA molecule complementary to all or a portion of said RNA template.

13. The method of claim 12, wherein said incubating step (b) comprises incubating said mixture at a temperature from about 40°C to about 80°C.

14. The method of claim 11, further comprising incubating said one or more cDNA molecules under conditions sufficient to make one or more double stranded cDNA molecules.

15. A method for amplifying a nucleic acid molecule, said method comprising

- (a) mixing an RNA template with a composition comprising one or more of the polymerases of claim 1 or claim 2 and one or more DNA polymerases to form a mixture; and
- (b) incubating said mixture under conditions sufficient to amplify a DNA molecule complementary to all or a portion of said RNA template.

16. The method of claim 15, wherein said incubating step (b) comprises

- (a) incubating said mixture at a temperature and for a time sufficient to make a DNA molecule complementary to all or a portion of said RNA template; and
- (b) incubating said DNA molecule complementary to said RNA template at a temperature and for a time sufficient to amplify said DNA molecule.

17. The method of claim 16, wherein said incubating step (a) comprises incubating said mixture at a temperature from about 40°C to about 80°C.

18. The method of claim 16, wherein said incubating step (b) comprises thermocycling.
19. The method of claim 18, wherein said thermocycling comprises alternating heating and cooling of the mixture to amplify said DNA molecule.
20. The method of claim 19, wherein said thermocycling comprises alternating from a first temperature range of from about 90°C to about 100°C, to a second temperature range of from about 45°C to about 75°C.
21. The method of claim 20, wherein said second temperature range is from about 60°C to about 75°C.
22. The method of 18, wherein said thermocycling is performed greater than 5 times.
23. The method of claim 15, wherein said DNA polymerases are thermostable DNA polymerases.
24. The method of claim 23, wherein said thermostable DNA polymerases are selected from the group consisting of *Tne*, *Tma*, *Taq*, *Pfu*, *Tth*, *Pwo*, *Tfl*, and mutants, variants and derivatives thereof.

25. The method of claim 15, wherein said DNA polymerases comprise a first DNA polymerase having 3' exonuclease activity and a second DNA polymerase having substantially reduced 3' exonuclease activity.

26. The method of claim 25, wherein said DNA polymerase having 3' exonuclease activity is selected from the group consisting of *Pfu*, *Pwo*, *Tne*, *Tma*, and mutants, variants and derivatives thereof.

27. The method of 25, wherein said DNA polymerase having substantially reduced 3' exonuclease activity is selected from the group consisting of *Taq*, *Tfl*, *Tth*, and mutants, variants and derivatives thereof.

28. The method of claim 15, wherein the unit ratio of said polymerases of claim 1 or claim 2 to said DNA polymerases is from about 0.25:1 to about 16:1.

29. The method of claim 15, wherein said mixture further comprises one or more nucleotides.

30. The method of claim 29, wherein said nucleotides are deoxyribonucleoside triphosphates or derivatives thereof, or dideoxyribonucleoside triphosphates or derivatives thereof.

31. The method of claim 30, wherein said deoxyribonucleoside triphosphates are

selected from the group consisting of dATP, dUTP, dTTP, dGTP, and dCTP:

32. The method of claim 15, wherein said mixture further comprises one or more oligonucleotide primers.

33. The method of claim 32, wherein said primer is an oligo(dT) primer.

34. The method of claim 32, wherein said primer is a target-specific primer.

35. The method of claim 32, wherein said primer is a gene-specific primer.

36. The method of claim 15, wherein said nucleic acid molecule is amplified by PCR, NASBA, TMA, 3SR, or SPSR.

37. A kit for synthesizing or amplifying a DNA molecule comprising one or more of the polymerases of claim 1 or 2.

38. The kit of claim 37, further comprising one or more deoxyribonucleoside triphosphates. ]

39. A method of preparing one or more cDNA molecules from one or more RNA templates, comprising:

(a) mixing one or more RNA templates with a purified thermostable template-dependent DNA polymerase from the species *Bacillus stearothermophilus* comprising reverse transcriptase activity in the presence of magnesium ions at a concentration of at least 1 mM and in the substantial absence of manganese ions to form a mixture; and

(b) incubating said mixture under conditions sufficient to synthesize one or more cDNA molecules complementary to all or a portion of said one or more templates.

40. The method of claim 39, wherein said polymerase is derived from *Bacillus stearothermophilus* strain ATCC number 12980.

41. The method of claim 39, wherein said polymerase further comprises 3'-5' exonuclease activity and 5'-3' exonuclease activity.

42. The method of claim 39, wherein said polymerase is modified or mutated to reduce or eliminate 3'-5' exonuclease activity.

43. The method of claim 39, wherein said polymerase is modified or mutated to reduce or eliminate 5'-3' exonuclease activity.

44. The method of claim 39, wherein said magnesium ion concentration is about 1 mM to about 10 mM.

45. The method of claim 39, wherein the source of said magnesium ions is a magnesium-containing buffer.

46. The method of claim 39, wherein the source of said magnesium ions is a magnesium-containing salt.

47. The method of claim 45, wherein said magnesium-containing salt is selected from the group consisting of magnesium chloride, magnesium sulfate, and magnesium acetate.

48. The method of claim 39, wherein said mixture further comprises betaine (MASTERAMP PCR ENHANCER).

49. The method of claim 39, wherein said incubating step (b) comprises incubating said mixture at a temperature and for a time sufficient to make a DNA molecule complementary to all or a portion of said RNA template.



50. The method of claim 39, further comprising incubating said one or more cDNA molecules under conditions sufficient to make one or more double stranded cDNA molecules.

51. A method for amplifying a nucleic acid molecule, said method comprising

(a) mixing an RNA template with a composition comprising a purified thermostable template-dependent DNA polymerase from the species *Bacillus stearothermophilus* comprising reverse transcriptase activity in the presence of magnesium ions at a concentration of at least 1 mM and in the substantial absence of manganese ions to form a mixture; and

(b) incubating said mixture under conditions sufficient to amplify a DNA molecule complementary to all or a portion of said RNA template.

52. The method of claim 51, wherein said nucleic acid molecule is amplified by RT-PCR, NASBA, TMA, 3SR, or SPSR.

53. The method of claim 51, wherein said mixture further comprises one or more oligonucleotide primers.

54. The method of claim 53, wherein said primer(s) is selected from the group consisting of an oligo(dT)

primer, a target-specific primer, and a gene-specific primer.

55. The method of 51, further comprising a second DNA polymerase having 3' exonuclease activity and a third DNA polymerase having substantially reduced 3' exonuclease activity.

56. The method of claim 55, wherein the unit ratio of said polymerase in claim 1 to said second and third DNA polymerases is from about 0.25:1 to about 16:1.

57. A kit comprising, in a packaged form, separate containers comprising:

(a) a purified thermostable template-dependent DNA polymerase from the species *Bacillus stearothermophilus* comprising reverse transcriptase activity; and

(b) a solution that provides magnesium ions at a concentration of at least 1 mM and in the substantial absence of manganese ions.

58. The kit of claim 57, wherein said polymerase is a mutated or truncated form of *Bacillus stearothermophilus*.